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Perspective

Protein Homeostasis and Aging: Taking Care of Proteins From the Cradle to the Grave

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All cells count on precise mechanisms that regulate protein homeostasis to maintain a stable and functional proteome. Alterations in these fine-tuned mechanisms underlie the pathogenesis of severe human diseases including, among others, common neurodegenerative disorders such as Alzheimer's or Parkinson's disease. A progressive deterioration in the ability of cells to preserve the stability of their proteome occurs with age, even in the absence of disease, and it likely contributes to different aspects of "normal" aging. A group of experts in different aspects of the biology of aging met recently to discuss the implications of altered protein homeostasis in aging, the current gaps in our understanding of the mechanisms responsible for proteome maintenance, and future opportunities for discovery in this area. We summarize here some of the key topics and main outcomes of the discussions.

Key Words: Proteases—Chaperones—Protein folding—Protein degradation—Proteotoxicity—Cellular homeostasis.

Protein Homeostasis and Cellular Quality Control

Three cellular machineries—translation, folding or assembly, and clearance—act coordinately to maintain the stability of the proteome and to ensure its continuous renewal under normal conditions (1–4). When protein damage occurs, these cellular machineries triage to repair damage by either refolding—if possible—or degrading the damaged proteins to prevent the accumulation of these toxic products inside the cell. Consequently, protein homeostasis results from the coordinated action of the quality control systems and the systems that govern each of the different processes that proteins undergo inside cells, including synthesis, assembly or disassembly, trafficking, and translocation to different intracellular compartments.

Altered protein handling has been implicated as a basis for a large number of common human diseases known as protein conformational disorders (1,5). Intracellular accumulation of abnormal proteins, in the form of protein inclusions and aggregates, and dysfunction of the quality control mechanisms are common in all these disorders. Similar features, although at slower pace, are also observed in "normal" aging cells, supporting the idea that alterations in protein homeostasis occur with age. However, the nature and extent of these alterations, its consequences for cellular functioning, and the contribution of these changes to different aspects of the phenotype of aging are, for the most part, unknown. We discuss here the current understanding of protein homeostasis in the context of aging, and delineate possible experimental approaches that could be applied in the near future to resolve these gaps of knowledge. The most pressing needs fall into three main categories: (a) the need to define the right homeostatic balance, (b) the need to understand the age-dependent changes in the machineries responsible for cellular homeostasis, and (c) the need to determine the types and consequences of these age- and disease-associated changes on the proteome. We then summarize the main conclusions reached in each of these points and comment on the possible future translational potential of some of the recent discoveries in protein homeostasis.

It Is All About Balance

Although the existence of defined intracellular systems responsible for protein handling (translation machinery, chaperones, proteolytic systems, repair enzymes) has been known for some time, and their molecular components have been the subject of intensive investigation as individual units, there is now common agreement on the importance of analyzing these systems in the context of a balanced complex network that preserves cellular functionality.

Understanding the mechanisms that govern the codependence of these systems could offer new explanations to cell type–dependent differences in the ability to respond to stressors, or help to discriminate the reasons behind altered cellular stasis in aging. The functional interdependence of these systems could explain, for example, why a primary defect in

protein folding can have a negative effect on the efficiency of the translation machinery, but also on the activity of clearance mechanisms, which often get clogged when the amount of misfolded proteins surpasses their capability. Likewise, defective function of the systems responsible for the removal of altered proteins leads to their accumulation in different cellular compartments that in turn alters the activity of other components of the quality control systems (1,5,6).

The interdependence of these systems explains the need to maintain them in continuous balance and provides a reason why any cellular event or intervention that causes an imbalance often has major consequences on cellular homeostasis. Despite balance among systems being identified as critical to understanding protein homeostasis, there are many unanswered questions. Is the "right balance" universal? Can the "set point" change to adapt to different cellular conditions? Does homeostatic stasis change with age? Is this a primary change or does it result from cellular adaptation to other age-related changes? If the concept of adaptive equilibrium is validated, dissecting the mechanisms behind this adaptation or cellular reprogramming to a different equilibrium set point could offer novel means to modulate protein homeostasis both under physiological conditions and also in those pathologies resulting from protein mishandling. However, the major limitation in this respect is our current inability to define the "right balance" for each cell type, where we should set the baseline for stasis, and how many degrees of separation from this baseline leads to cellular toxicity or functional loss.

There is general agreement that the most efficient approach to obtain a comprehensive understanding on cellular stasis and to help establish the cellular baseline of this process will require a systematic analysis in many different settings and cell types. Gathering simultaneous functional information on the synthetic, folding, and clearance machineries would allow comparative analyses among cell types, tissues, and organisms that should clarify whether there is a universal baseline, and whether the right balance changes with cellular conditions (ie, proliferative capability, exposure to stress, aging). A critical point in these proposed systematic analyses is the need for highly reliable readouts of the functional status of the different network systems involved in protein homeostasis. Approaches that offer functional information over time and in multiple tissues of the same organism will have better chances of providing an integrated view of the homeostatic mechanisms and of the principles behind the regulation of their coordinated function.

Learning About the Machineries That Regulate Homeostasis

As aforementioned, an essential component for future success in the analyses of protein homeostatic cellular networks is the use of analytical tests that can provide accurate, real-time information on the function of the individual systems integrated in the network.

The protein biogenesis and quality control machineries—with the notable exception of repair systems—are well defined, and methods to study their dynamics and functionality under different conditions have been implemented and validated. However, there is a general lack of information on the changes of those systems with age. Descriptive comparative analyses in young and old organisms of the levels of individual components of the systems, or of their activity in vitro, are not going to suffice at this point. The peculiarities of the cellular environment in which those systems have to perform their tasks in old organisms have a major impact on their functionality and these environmental factors are not easily reproduced using *in vitro* models. For example, measurement of the activity of an enzyme from young and old tissue samples using artificial substrates may offer very limited information on how that enzyme is handling real substrates encountered in the aging cellular environment. Consequently, there is an urgent need to move the studies on the biosynthetic and quality control mechanisms to the level of whole cells and organisms.

On a positive note, during recent years there have been major advances in methods and procedures in the cell biology field, many of them based on novel imaging technologies, that could be implemented in the study of an aging cell (7,8). Although many of these procedures still rely on tracking a reporter protein, rather than multiple endogenous proteins as would be preferable, some of the added advantages of these approaches are that they allow to (a) compare the behavior of the same model protein in many different cell types and tissues, (b) learn about cell-to-cell variations even when analyzing the same cell type, (c) perform kinetic analyses (in particular because of the introduction of photoswitchable and photoactivable proteins), (d) study simultaneously the interaction of the model proteins with different elements of the protein homeostatic network by introducing markers for those systems along with the reporter, and (e) analyze the same individual organism at different times, or before and after performing particular interventions through the current development of procedures for in-depth imaging and magnetic resonance approaches in live animals.

One additional aspect that deserves future attention is the distinctive ways by which different intracellular compartments handle proteins. Folding, repair, and degradation of proteins in the cytosol are well-understood processes, and the main molecular players in each of these processes have been extensively analyzed (9–11). Likewise, our understanding of endoplasmic reticulum (ER) proteotoxicity has also grown exponentially in recent years as a result of the detailed characterization of ER stress, the unfolding protein response, and its associated degradation systems (12,13). However, there is still relatively limited information on protein homeostasis in other cellular compartments. The concept of a mitochondrial unfolded protein response has just emerged (14), and similar responses are still unknown for other cellular compartments. How does the nucleus handle protein aggregation, something

quite common in many neurodegenerative disorders? Are resident lysosomal proteins amenable to refolding if they are altered, or is degradation their only fate? Damaged proteins often end in the lysosomal compartment for degradation, so does lysosomal proteotoxicity occur if levels of damaged proteins exceed the capacity of the lysosomal proteases? Furthermore, how does proteotoxicity in one compartment affect the others? Can repair and refolding enzymes be redirected from one compartment to another when the need arises? What mediates the cross talk among the surveillance mechanisms in the different intracellular compartments; and, overall, how are these different systems affected in aging? Are some repair systems more susceptible to the aging process, or if there is a sequential order in which some of these systems are targeted, can they be compensated for by the remaining ones? Implementation of similar procedures to the ones previously mentioned, combining reporters resident in different cellular compartments and performing systematic analyses in different tissues and under different conditions, would be one of the possible ways to answer these questions.

Do We Know Enough About the "Customers"?

The use of reporter proteins while providing unique insights into the functional status of the cellular surveillance systems, nevertheless, does not provide information on the fate of specific endogenous intracellular proteins. Consequently, a direct analysis of the status of the proteome in aging organisms is essential to fully understand the consequences of the loss of protein homeostatic ability with age.

In that respect, there is an extensive literature reporting different types of age-related posttranslational changes in individual proteins that contribute to changes in their physical properties (eg, solubility) and function (15). It is also widely accepted that aggregated proteins accumulate in many postmitotic cells with age, and some frequent components of those aggregates have also been identified. The proteins that have been individually tracked in these studies are often selected based on their abundance, the availability of tools for their study, or their association with particular disorders or cellular changes. Therefore, the information gathered on these selected proteins is far from providing a global view of the status of the proteome. What fraction of the proteome is at risk when the systems responsible for protein homeostasis fail? Are the proteins particularly susceptible to global changes or with an intrinsic propensity to aggregate the same in all tissues? How do such aggregation-prone proteins affect the balance of the folding and clearance mechanisms? A recent report has identified the presence of a pool of marginally stable or folding-defective proteins that can modify aggregation and toxicity of pathogenic proteins in a disease background (16). This pool of metastable proteins is particularly susceptible to minimal changes in chaperone availability. Whether this protein pool is constant and universal for all cells and tissues, or if the pool changes depending on the cell type, cellular conditions, age, or even among individuals—thus explaining differences in susceptibility to proteotoxicity—requires further investigation. Analyses of that nature will need development of new models in which all proteins, or at least subsets of cellular proteins with different propensity to aggregate, are followed in the same study model under different conditions over time.

Besides this intrinsic instability, there are also numerous examples of protein modifications, imposed by changes in the cellular environment (eg, oxidation, glycation, nitration) that can alter protein stability. Although our understanding of the biochemistry behind these modifications has advanced, there is still a major lack of information on their biological consequences. What is the critical determinant of cellular toxicity—the extent of damage, the type of damage, or the primary site at which it occurs? Is damage random or protein-specific and does protein damage always lead to loss of function? Is the effect of cumulative damage additive? Do the consequences of the same type of damage change with age as a result of other changes in the proteome? Future studies will need to take into account the distinction between true damage and normal functional modifications, as a growing number of reports show that changes previously associated with protein damage are in fact used to increase the functional diversity of proteins. For example, protein oxidation is used extensively by cells as part of their signaling mechanisms.

As aforementioned, in contrast to the relatively well-understood mechanisms used by cells to get rid of irreversibly damaged proteins, with the notable exception of a few enzymes, there is almost a complete lack of information on the systems plausibly responsible for protein repair. In support of the importance of the repair systems in aging, recent studies have shown that changes in the levels of these few known repair enzymes have major consequences on life span in invertebrates. One of the reasons that could contribute to the slow pace at which we are learning about these systems could be attributed to the lack of real-time studies on proteostasis. To analyze reversibility of damage, the same protein needs to be tracked through time, before and after the damage occurs, thus making essential the implementation of reporters of different types of protein damage that preferably could be used in a whole organism. The current development of fluorescent probes sensitive to particular modifications should have a positive impact on this type of studies.

The dramatic increase in analytical power and sophistication of proteomic procedures could also be of major use to resolve some of these questions related to protein damage inside cells. However, processing the amount of information generated by proteomic analyses of this type could become a real challenge. Isolated proteomic studies have a limited value as they offer only a snapshot of the cell. Instead, time course analysis in the same organism are necessary to understand the dynamics that govern the changes in the proteome with age. In addition, multiple tissues in the same organism should be included in these

analyses because there is growing evidence that changes in the proteostasis networks, for example, in the central nervous system, have an impact on peripheral proteostasis (17). Thus, this becomes a typical example where a systems biology approach could be particularly advantageous to help integrate all the information gathered through these studies (see also article by West & Bergman, [8]).

Therapeutic Implications of the New Findings in Protein Homeostasis

From the aforementioned brief summary of the status of our understanding of protein homeostasis, it is clear that there are a number of critical unanswered questions. Fortunately, the renewed interest in this area, in particular in the context of aging and age-related disorders, should help provide the momentum to drive the necessary growth in this field. Thus, it does not seem that we will have to wait much longer before manipulations aimed at modifying the proteostasis networks could be implemented as anti-aging interventions.

The beauty of a highly integrative network is that small readjustments of a single system seem to have global beneficial effects. For example, improving chaperone availability, which in theory would only affect the folding arm of the system, can restore proteostasis (16,19). Similarly, preventing the age-dependent decline of only one of the multiple mechanisms of protein clearance has been shown sufficient to improve protein homeostasis and organ function (20). In both examples, blind readjustment of the system has proven efficient, suggesting that the proteostasis networks have a propensity to naturally rebalance. Although further studies of this type are required, the tendency of the proteome to naturally reorganize seems to be universal, as these initial interventional studies have been done in very different model organisms with similar results. This observation, along with the fact that modification in one system seems to reset all the other systems involved in protein homeostasis, could open new basis for future therapeutic interventions.

Further attention should also be paid to the possible therapeutic value of preconditioning or what could be conceived as hormesis of the proteostasis networks. Different reports support a positive effect of previous exposure to small doses of a stressor on the response to cellular stress (21). Preconditioning has shown an enhancing effect on chaperones and proteolytic systems of old organisms. Whether this is a universal phenomenon in all cells and for all types of stress and whether this could be exploited as part of a global prophylactic plan to better prepare old organisms for conditions associated to proteotoxicity are part of the future challenges.

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